ON THE SIGNIFICANCE OF INTRACYTOPLASMIC INCLUSIONS IN THE URINARY SEDIMENT

MYRON R. MELAMED, M.D., AND WANDA H. WOLINSKA

From the Cytology and Pathology Laboratories, Memorial Hospital for Cancer and Allied Diseases, New York, N.Y.

In a recent cytologic and pathologic study of early urinary bladder carcinoma,[1] we noted peculiar eosinophilic intracytoplasmic bodies of unknown significance within degenerating cells of the urinary sediment (Fig. 1). Similar bodies were described by Bolande[2] in urine from children with measles and other viral diseases. He generously afforded us the opportunity to examine some of his preparations and to compare them with our own. The cytoplasmic bodies in his and our material appeared to be identical morphologically. It thus seemed desirable to review more material, largely from an adult population, to look for possible relationships between these inclusion-bearing cells and specific disease states.

MATERIAL AND METHODS

Papanicolaou-stained urine sediment smears[1] from 500 consecutive patients, observed subsequent to January 1, 1959, were taken from the files of the Cytology Laboratory of the Memorial Hospital, New York. The smears were re-examined for the specific purpose of noting cells with eosinophilic, intracytoplasmic bodies. These were marked and counted in each preparation. Over-all epithelial cellularity, inflammation, blood, and crystals were noted in each of the smears. If fewer than 5 cells with inclusions were found in a specimen, it was classified as having "few"; if 5 to 20 cells were found, it was classified as "moderate"; if more than 20 cells were present, the designation was "many." Follow-up specimens were examined in a few instances, as indicated. Clinical data were obtained from the hospital charts after the urine smears were examined. A few cases were discarded because insufficient information was available, and these were replaced by others, consecutively taken.

MORPHOLOGY

Once recognized, the intensely staining intracytoplasmic eosinophilic bodies were easily identifiable (Fig. 1). They contrasted sharply with the usual pale gray cytoplasm of exfoliated cells in urinary sediment. When well formed, they were characteristically round or ovoid and measured as much as 12 to 15 μ in diameter. More commonly, they ranged from barely resolvable granules (Fig. 2) to a diameter of about 10 μ (Fig. 3). The larger ones were characteristically single; the smaller,

Presented in part at the Eighth Annual Meeting of the Intersociety Cytology Council, September 24, 1960, Chicago, Ill.

Accepted for publication, January 12, 1961.

more frequently multiple. There was also variation in configuration. Irregularly ovoid or club-shaped forms were not uncommon; more rarely they were rod-shaped (Fig. 4), curvilinear (Fig. 5), or even branching (Fig. 6).

We have never seen these inclusions within nuclei. Almost always the affected cell was degenerated, and often no longer identifiable as to type. Most commonly the nuclei were pyknotic (Figs. 1 and 2) or showed marked chromatin clumping (Fig. 7) and karyorrhexis (Fig. 8). Occasionally there was chromatin fading without loss of nuclear membrane staining. This resulted in "ring" nuclei with, perhaps, a clump or two of peripherally placed chromatin (Fig. 3). Well-formed inclusions were seen occasionally within anuclear cytoplasmic remnants (Fig. 9); these, in some instances, were multiple and varied in size, appearing no different from those in nucleated cells. Only rarely were inclusions found within relatively well preserved epithelial cells (Fig. 10). Neoplastic (Fig. 6) as well as non-neoplastic cells were affected. Oil immersion magnification failed to reveal any internal structure within the inclusions (Fig. 11).

It was not possible to identify stages in the development of the inclusions conclusively. The degree of nuclear degeneration did not correlate with alterations in their size, number, or configuration. Certain cells (Fig. 12) led to the impression that the inclusions were formed by condensation of an amorphous eosinophilic-staining cytoplasmic substance such as is noted in occasional cells of the urinary sediment. Yet, minute intracytoplasmic bodies were seen in cells which did not exhibit diffuse eosinophilia (Fig. 2). Occasionally it appeared that larger inclusions formed by confluence of smaller ones (Fig. 13), perhaps explaining why the large ones were characteristically single. Bladder biopsy specimens which showed intracytoplasmic inclusions within intact epithelium likewise offered few clues to their mode of origin and development (Fig. 14).

Results

Incidence

Altered cells containing the intracytoplasmic bodies were identified in 217 (43 per cent) of the 500 consecutive cases examined (Table I). They were more commonly found in voided than in catheterized urine (Table II) and more frequently in men than in women (Table III). There was a disproportionately greater number of catheterized specimens from women (80 per cent) than men (41 per cent), accounting for some of the over-all difference in incidence between the sexes. However, even in the catheterized specimens there was a higher incidence of in-

clusions in men than in women (Table III). Interestingly enough, catheterization appeared to be associated with a lower incidence of inclusion-bearing cells in men only (Table III). This will be further discussed below. The patients investigated included a few children but were largely an adult population. There seemed to be a slight increase in incidence of inclusion-bearing cells in the oldest age groups (Table IV).

TABLE I

INCIDENCE OF INCLUSIONS IN URINE SPECIMENS
(Total cases examined: 500)

Inclusions	Cases	
Few (1 to 4)	135	
Moderate (5 to 19)	52	
Many (20+)	30	
Total:	217	(43%)

TABLE II

INCLUSIONS IN VOIDED VS. CATHETERIZED URINE *

Cells with inclusions	Cath.†	Void.
None	146	76
Few (1 to 4)	45	46
Moderate (5 to 19)	16	17
Many (20+)	13	9
Incidence of inclusions	34%	49%

* Information whether catheterized or voided available in 368 cases.

† Includes 13 specimens of ureteral urine.

TABLE III

INCIDENCE OF INCLUSIONS IN MEN AND WOMEN

	All specimens		Bladder urine only * (catheterized)	
	M	F	M	F
Total examined	297	203	81	126
Incidence of inclusions	50%	33%	41%	32%

* Thirteen specimens of ureteral urine not included.

TABLE IV

AGE OF PATIENTS AND OCCURRENCE OF INCLUSIONS

	Age (yrs.)		
	<40	40–60	60+
Total patients	48	173	279
Inclusions present	32%	40%	48%

Urinary Tract Disease

An effort was made to relate the inclusion-bearing cells to urinary tract disease of various types (Table V). The cells were most commonly found among patients with primary bladder cancer and somewhat less commonly among those with no urinary tract abnormality. This difference in incidence was relatively small and at least partly explainable by differences in cellularity and evidences of inflammation in the urine sediment.

There were 30 specimens containing many (over 20) inclusion-bearing cells. Even in these there was no apparent correlation with specific urinary tract disorder (Table V). Two of the 30 had no urinary tract disease at all; in one there was a pulmonary granuloma; in the

other, arthritis. A second examination was possible in 12 of the 30, 1 day to 9 months later. In only 4 was there a persistence of many (over 20) inclusion-bearing cells; in 2 of these a 7-month time interval had elapsed. Only 1 of the 12 specimens had no inclusion-bearing cells; in this instance the second specimen was taken 2 days after the first and followed resection of a bladder papilloma.

TABLE V

INCIDENCE OF INCLUSIONS IN VARIOUS DISEASE STATES

	Total cases	Inclusions	
		Present	Many (No. of cases)
No disease	72	37%	2
Non-neoplastic disease	196	42%	14
Benign bladder papillomas	46	44%	4
Bladder carcinoma	115	49%	8
Other cancers	71	44%	2

TABLE VI

INCIDENCE OF INCLUSIONS AFTER URINARY TRACT MANIPULATION (Total cases: 89)

Inclusions	No. of cases
None	45
Few (1 to 4)	25
Moderate (5 to 19)	14
Many (20+)	5
Total with inclusions	49%

Systemic Disease

More than half the patients in this series were over 60 years old, and they suffered the degenerative diseases common to this age group. It was difficult to assess the importance of arteriosclerosis except by equating it with age. As noted, inclusions were somewhat more common in the older age groups (Table IV). There were 29 patients with diabetes. Of these, 14 were found to have inclusion-bearing cells in their urine. This paralleled the incidence in the nondiabetic group. Hepatitis was present in 4 patients at the time of urine examination, or became apparent shortly thereafter. Inclusions were present in 2 of these. One patient, presumed to have viral neuropathy, had no inclusions in the urine. No other viral disorders were clinically apparent in the other patients.

There was no consistent pattern of systemic disease manifest in the cases with inclusion-bearing cells in the urine.

Local Factors

Marked degeneration was almost invariably evident in inclusion-bearing cells. Yet, in evaluating factors generally associated with increased numbers of degenerating cells in the urinary sediment we were again unable to find any convincing leads. Intracytoplasmic bodies were

present in 48 per cent of 96 patients with a history of prior radiation therapy to the pelvis or abdomen for various reasons, or who had had chemotherapy. In the urine of an additional 23 patients currently receiving radiation therapy, 12 (52 per cent) had inclusions. Thus, neither recent nor remote radiation, nor systemic chemotherapy appeared to alter the incidence of the inclusions. Manipulation of the bladder within the preceding 2 months (cystoscopy, biopsy, fulguration, silver nitrate instillation, indwelling or repeated catheterization, or retrograde pyelogram) also had no apparent bearing on the incidence of the bodies (Table VI).

The frequency of inclusions was slightly increased in cases with evidence of moderate inflammation, as judged by the presence of leukocytes in the smears (Table VII), but cases with marked inflammation did not confirm this relationship. Finally, an attempt was made to correlate the bodies with over-all epithelial cellularity in the smears. As might be anticipated, when epithelial cells were sparse, the inclusions were less likely to be found (Table VIII). Abundant cellularity, however, was not accompanied by an increase over the group as a whole.

TABLE VII

COINCIDENCE OF INCLUSIONS AND INFLAMMATION

Inflammation	Total cases	Inclusions present
None	261	33%
Slight	122	51%
Moderate	71	60%
Marked	46	50%

TABLE VIII

COINCIDENCE OF INCLUSIONS AND EPITHELIAL CELLULARITY

Cellularity of urine *	Total cases	Inclusions present
+	111	30%
++	254	44%
+++	99	55%
++++	36	50%

* + to ++++ indicates increasing cellularity from very sparse to abundant.

The presence of red blood cells, crystals, spermatozoa, and parasites (Trichomonas) had no influence on the occurrence or detection of the inclusion-bearing cells.

DISCUSSION

Despite the usual marked degeneration of affected cells, it was often possible to identify them as of epithelial origin. Indeed, inclusions were occasionally seen in clearly malignant neoplastic epithelial cells from patients with bladder carcinoma (Fig. 6). By carefully examining histologic sections of bladder biopsy specimens in selected cases we have also been able to demonstrate inclusions within cells in the intact epithelium (Fig. 14).

Inclusion-bearing cells were more frequent in voided than in catheterized urine from men, but not women (Table III), suggesting that some also originated in the male urethra (Fig. 15). In fact, we found them in urethral washings from a male patient following total cystectomy. They were also found in urine obtained by ureteral catheterization. Seminal fluid was not examined for inclusion bodies, but similar inclusions have been encountered in the epithelium of prostatic carcinoma.

Inclusion-bearing cells were found quite regularly in urine specimens from adults, without regard to any specific disease process. In fact, they were also present with almost as high an incidence in well subjects. As might be expected, they were less likely to be found in specimens with only sparse cellularity, and thus were somewhat less common in the healthy individuals.

Similar eosinophilic intracytoplasmic bodies have also been described in degenerating exfoliated cells in sputum. Papanicolaou,[3] who first drew attention to them, found them in the sputum from a number of patients with a variety of acute and chronic respiratory diseases. He was impressed by a relatively frequent association with bronchogenic carcinoma and subsequently reported a case in which this alteration in sputum led to further studies on a patient who later developed lung cancer.[4] In detailed clinical investigations of this phenomenon, Pierce and her colleagues [5,6] associated the inclusions with acute viral respiratory disease and, more specifically, adenovirus and influenza.

In a footnote to his original description of the inclusions in sputum,[3] Papanicolaou mentioned seeing similar changes in urinary, endocervical and mammary cells. We can confirm these observations; moreover, we found identical inclusion-bearing cells in pleural and ascitic fluid and in gastric and colonic washings. If the inclusions as they appeared in the urine were the result of viral infection, they would seem to be due to viruses universal in distribution among the adult population and apparently unassociated with clinical manifestations. Our observations strongly suggest that the inclusions are nonspecific in nature but probably reflect a degenerative cellular alteration.

Summary

Urine specimens procured from 500 consecutive adults with and without urinary tract disease were re-examined in order to establish the incidence and significance of exfoliated inclusion-bearing cells. There was no correlation with any specific disease state, either a systemic process or one localized to the urinary tract. Our observations suggest that morphologically striking, eosinophilic, intracytoplasmic bodies,

which resemble viral inclusions, reflect a degenerative cellular change unassociated with a specific clinical ailment.

References

1. Melamed, M. R.; Koss, L. G.; Ricci, A., and Whitmore, W. F. Cytohistological observations on developing carcinoma of the urinary bladder in man. *Cancer*, 1960, **13**, 67–74.
2. Bolande, R. P. Inclusion-bearing cells in the urine in certain viral infections. *Pediatrics*, 1959, **24**, 7–12.
3. Papanicolaou, G. N. Degenerative changes in ciliated cells exfoliating from the bronchial epithelium as a cytologic criterion in the diagnosis of diseases of the lung. *New York J. Med.*, 1956, **56**, 2647–2650.
4. Papanicolaou, G. N. Cellular changes in the development of pulmonary cancer as revealed by cytology: a case report. *Acta Unio internat. contra cancrum*, 1958, **14**, 479–484.
5. Pierce, C. H., and Hirsch, J. G. Ciliocytophthoria: relationship to viral respiratory infections of humans. *Proc. Soc. Exper. Biol. & Med.*, 1958, **98**, 489–492.
6. Pierce, C. H., and Knox, A. W. Ciliocytophthoria in sputum from patients with adenovirus infections. *Proc. Soc. Exper. Biol. & Med.*, 1960, **104**, 492–495.

[*Illustrations follow*]

Legends for Figures

All photographs were prepared from smears stained by the Papanicolaou method or tissue sections stained with hematoxylin and eosin.

Fig. 1. Transitional epithelial cell with peripheral pyknotic nucleus. The deeply eosinophilic, sharply defined, round intracytoplasmic body (gray in photograph) is actually larger than the nucleus. The adjacent cell is from relatively normal bladder epithelium. × 500.

Fig. 2. This cell, with a pyknotic nucleus, contains multiple (bright pink or orange) intracytoplasmic inclusions. Some appear as tiny granules and, though appearing gray in the photograph and difficult to see, are actually readily visible because of their intense eosinophilia. × 500.

Fig. 3. This single large round intracytoplasmic inclusion appears as a solid gray body (actually stains deep pink). Note the peripheral condensation of nuclear chromatin in this degenerating double-nucleated cell. × 500.

Fig. 4. A rod-shaped eosinophilic intracytoplasmic inclusion. Nuclear chromatin clumping indicates early cellular degeneration. × 500.

Fig. 5. The eosinophilic intracytoplasmic inclusion is curvilinear in this instance. Though it appears poorly defined in the photograph, it actually is readily apparent because of its pink staining. × 500.

Fig. 6. A huge, irregularly branching, rod-shaped intracytoplasmic inclusion within a cancer cell. This patient had epidermoid carcinoma of the bladder. × 500.

Fig. 7. Nuclear chromatin clumping is marked in this inclusion-bearing cell and probably would have progressed to karyorrhexis. The pink-staining intracytoplasmic body contiguous to the nucleus is readily distinguished by its striking eosinophilia. × 500.

Fig. 8. Karyorrhexis. A single, deeply pink-stained, round inclusion appears here as a large, gray, peripherally placed body. The many smaller, dense, irregular fragments are all nuclear debris. × 500.

Fig. 9. A single, well formed, brightly eosinophilic inclusion within an anuclear cytoplasmic remnant of a cell. × 500.

Fig. 10. A single, small but well defined, eosinophilic inclusion within the cytoplasm of a well preserved epithelial cell. × 500.

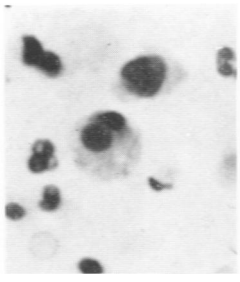
Fig. 11. A large, ring-shaped inclusion within the cytoplasm of a degenerating cell with a pyknotic nucleus. Even at this high magnification the inclusion body is homogeneous and structureless. × 1400.

Fig. 12. In occasional cells, such as this, tiny eosinophilic granules emerge from areas of diffuse eosinophilia within the cytoplasm. × 1400.

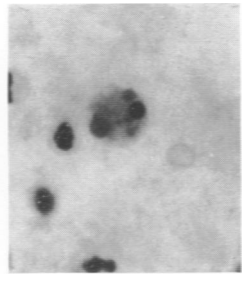
Fig. 13. This cell contains multiple small, deeply pink-staining inclusions which are apparently becoming confluent. The single nucleus is small, dark-staining, and peripheral. × 500.

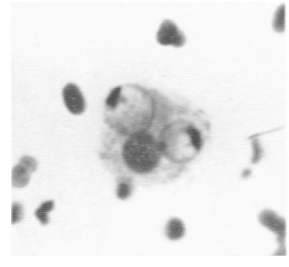
Fig. 14. Bladder biopsy specimen from a patient with many inclusion-bearing cells in the urinary sediment. Numerous round pink inclusions (which here appear gray) are readily apparent within the cytoplasm of cells in the intact epithelium. Hematoxylin and eosin stain. × 350.

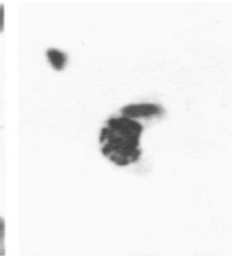
Fig. 15. Multiple, pink-staining intracytoplasmic inclusions (which appear here as pale gray) are readily distinguished from the darkly staining nuclear fragments in this degenerating squamous cell. × 500.



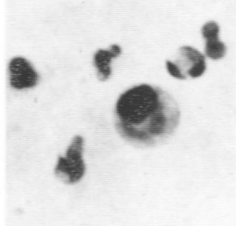
1, 2



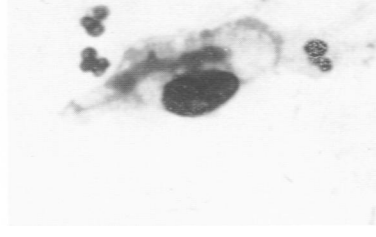


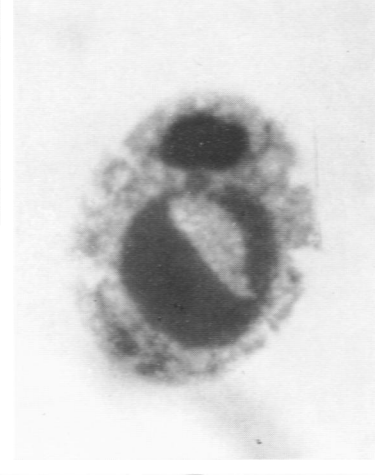


3, 4

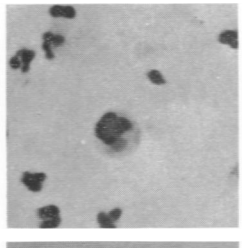


5, 6

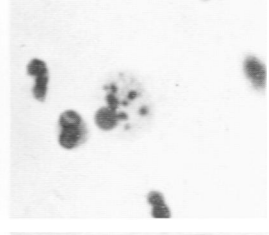


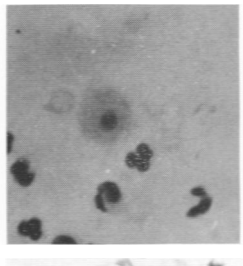


11

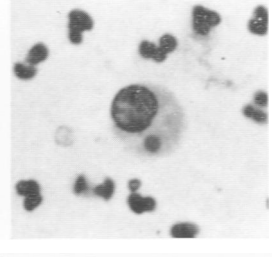


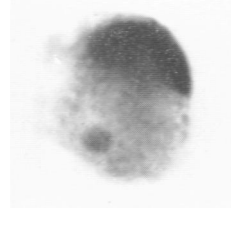
7, 8

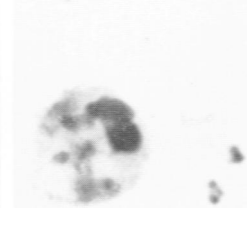




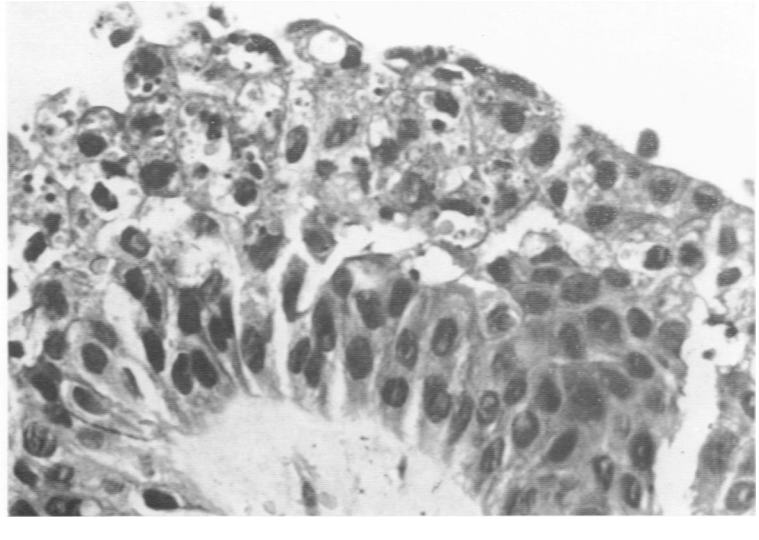
9, 10







12, 13



14

15